

## **IFNB/Interferon- $\beta$ regulates autophagy via a *MIR1-TBC1D15-RAB7* pathway**

Patrick Ejlerskov<sup>1,2#</sup>, David C. Rubinsztein<sup>2,3#</sup>, and Roger Pocock<sup>4#</sup>

<sup>1</sup>Biotech Research and Innovation Centre, Faculty of Health and Medical Sciences, University of Copenhagen, Ole Maaløes Vej 5, Copenhagen, Denmark

<sup>2</sup>Cambridge Institute for Medical Research (CIMR), Cambridge Biomedical Campus, The Keith Peters Building, University of Cambridge, Cambridge, CB2 0XY, UK

<sup>3</sup>UK Dementia Research Institute, Cambridge Biomedical Campus, Cambridge, UK

<sup>4</sup>Development and Stem Cells Program, Monash Biomedicine Discovery Institute and Department of Anatomy and Developmental Biology, Monash University, Melbourne, Victoria 3800, Australia

# Correspondence should be addressed to R.P. ([roger.pocock@monash.edu](mailto:roger.pocock@monash.edu)), D.C.R. ([dcr1000@cam.ac.uk](mailto:dcr1000@cam.ac.uk)), and P.E. ([patrick.ejlerskov@bric.ku.dk](mailto:patrick.ejlerskov@bric.ku.dk)). Lead contact is Roger Pocock

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## Abstract

Loss of IFNB/interferon- $\beta$  in mice causes a Parkinson disease-like phenotype where many features, including SNCA/ $\alpha$ -synuclein and MAPT/tau accumulation, can be attributed to a late-stage block in autophagic flux. Recently, we identified a mechanism that can explain this phenotype. We found that IFNB induces expression of *Mir1*, a microRNA that can reduce the levels of TBC1D15, a RAB GTPase-activating protein. Induction of this pathway decreases RAB7 activity and thereby stimulates macroautophagy/autophagy. The relevance of these key players is deeply conserved from humans to *Caenorhabditis elegans*, highlighting the importance of this ancient autophagy regulatory pathway.

## Main text

Autophagy is a pathway that enables degradation of cytoplasmic contents by wrapping them within double-layered vesicles called autophagosomes. These autophagosomes fuse with lysosomes, which facilitates degradation of their luminal contents. The transport and fusion steps are governed by numerous proteins, including RAB GTPases, such as RAB7, HOPS and SNARE proteins.

The accumulation of neurotoxic aggregation-prone proteins is a hallmark of many age-related neurodegenerative diseases, such as Parkinson disease, Alzheimer disease, Huntington disease, and amyotrophic lateral sclerosis. Mendelian mutations associated with many neurodegenerative diseases compromise autophagy, and these potentiate the accumulation of various aggregate-prone proteins, because such proteins, including mutant HTT (huntingtin), MAPT/tau and SNCA/ $\alpha$ -synuclein, are autophagic substrates. Autophagy is particularly important for the degradation of oligomeric and higher-order assemblies that characterize these neurodegenerative disease-causing proteins, as these are inaccessible to the proteasome, or chaperone-mediated autophagy.

Previously, we reported that mice lacking the *Ifnb1* (interferon beta 1, fibroblast) gene, an essential cytokine in anti-viral immune responses and T-cell regulation, develop a phenotype resembling Parkinson disease with the accumulation of intraneuronal SNCA and MAPT aggregates, and loss of dopaminergic neurons, associated with cognitive and motor disturbances. We found that lack of IFNB input causes a late-stage block in neuronal autophagy compatible with impaired autophagosome degradation. Conversely, treatment with recombinant IFNB promotes autophagy flux, and coherently we demonstrated that *in vivo* brain expression of *Ifnb* reduces SNCA-induced neurotoxicity in a rat model of Parkinson disease. However, it was not clear how IFNB regulates autophagy.

Recently, we identified a mechanism explaining how IFNB influences autophagy by focussing our studies on the microRNA, *mir-1*, which is depleted in a *Drosophila melanogaster* model of Alzheimer disease, and *MIR1*, which is reduced in the cerebrospinal fluid of patients with Parkinson disease [1]. microRNAs rapidly downregulate translation of multiple target genes by imperfect binding to motifs in their 3' UTR region. *MIR1* is a highly conserved miRNA and despite 600 million years of evolutionary distance, the sequence of the *miR-1/MIR1* seed region is fully conserved in worms and humans. Thus, we made use of a *C. elegans* *in vivo* model to ectopically express an aggregation-prone fragment of mutant HTT (HTT<sub>Q40</sub>), which causes Huntington disease-like pathology. Loss of *mir-1* leads to the formation of significantly more HTT<sub>Q40</sub> protein aggregates when compared to the wild-type worms, and expressing wild-type *mir-1* in mutant worms reduces the aggregation burden. Similarly, loss of *mir-1* in worms enhances exogenous SNCA aggregate formation and causes behavioral abnormalities. These defects are associated with our finding that *mir-1* is a positive autophagy regulator.

Consistent with the worm data, we found that overexpression of *MIR1* decreases mutant HTT exon 1 aggregation in HeLa cells, and this could be attributed to enhanced autophagic flux. From the TargetScan database we identified human TBC1D15 (TBC1 domain family member 15) and a *C. elegans* homolog TBC-7 as potential targets of *MIR1/mir-1*, both of which are putative negative regulators of autophagy. These proteins are RAB-GTPase activating proteins (GAPs), which promote termination signalling of RAB GTPases, and TBC1D15 targets RAB7, a known regulator of autophagy. Thus, we tested and confirmed that TBC1D15/TBC-7 is a genuine target of *MIR1/mir-1* using *C. elegans*- and human-specific reagents. For example, *MIR1* targets the 3' UTR of *TBC1D15* in HeLa cells and consequently reduces the amount of TBC1D15 protein.

Next, we investigated how TBC1D15 influences autophagy flux. Overexpression of TBC1D15 in HeLa cells causes a late-stage block in autophagy compatible with defective autophagosome degradation, and TBC1D15 knockdown mimics the autophagy induction observed with *MIR1* overexpression. The increase of autophagy induced by *MIR1* is blocked by overexpression of *TBC1D15* that lacks the endogenous 3' target sequence for *MIR1*. Finally, we showed that RAB7 is indeed a target of TBC1D15, as overexpression of TBC1D15 significantly reduces the amount of active GTP-bound RAB7. As expected, loss of RAB7 leads to a late-stage block in autophagy, and co-expression of a constitutive active RAB7<sup>Q67L</sup> mutant counteracts the TBC1D15-mediated autophagy block.

A previous study observed that IFNB regulates *MIR1* in hepatic cells. This provided the clue and impetus to test whether this cytokine could regulate autophagy and aggregate-prone protein clearance via the *MIR1*-TBC1D15-RAB7 pathway. We found that IFNB upregulates *Mir1* while reducing the amount of TBC1D15 protein in primary cortical neuron cultures from mice, and in HeLa cells, where the effect of the cytokine on TBC1D15 levels depends on the presence of an intact 3' UTR *MIR1* binding site. The effects of IFNB on autophagic flux and mutant HTT accumulation depend on *MIR1* and TBC1D15, as they are abrogated by a *MIR1* hairpin inhibitor or TBC1D15 overexpression.

Collectively, our data provide new mechanistic insight into how IFNB promotes autophagy via *MIR1*-TBC1D15-RAB7, thereby limiting accumulation of aggregation-prone proteins such as SNCA and HTT (Figure 1). The deep evolutionary conservation from worm to human emphasizes the biological importance of *MIR1*, and it will be interesting to explore the possible therapeutic potential of this pathway.

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## **References**

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**Figure 1.** Schematic overview. **(A)** IFNB binds to IFNAR1/IFN- $\alpha/\beta$ -receptor and activates downstream signaling **(1)** including transcription of *MIR1* **(2)**, which binds to the 3' UTR of *TBC1D15* and thereby repress its translation **(3)**. TBC1D15 is a GTPase activating protein (GAP), which shuts down the activity of RAB7 by promoting GDP-binding. Thus, with low levels of TBC1D15, the abundance of active GTP-bound RAB7 will be favored due to guanine nucleotide exchange factor (GEF) activity **(4)**. Protein aggregates are targeted by autophagy cargo receptors and subsequently couple to LC3-II in the phagophore membrane **(5)**, which eventually forms a vesicular autophagosome **(6)**. The autophagosomes fuse with lysosomes for clearance of the protein aggregates in a process promoted by active RAB7 **(7)**. **(B)** If TBC1D15 translation is increased, e.g., by low *MIR1* **(8)** this shuts down RAB7 activity **(9)**, and under stress conditions neurotoxic protein aggregates will form due to reduced autophagy **(10)**.

Figure 1

